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(71) Applicant (for all designated States except US):
LAMIRSA S.A. [ES/ES]; Pol. Industrial Can Par-
ellada, Calle Geminis, n° 4, E-08228 Les Fonts de
Terrassa/Barcelona (ES).

(72) Inventors: and

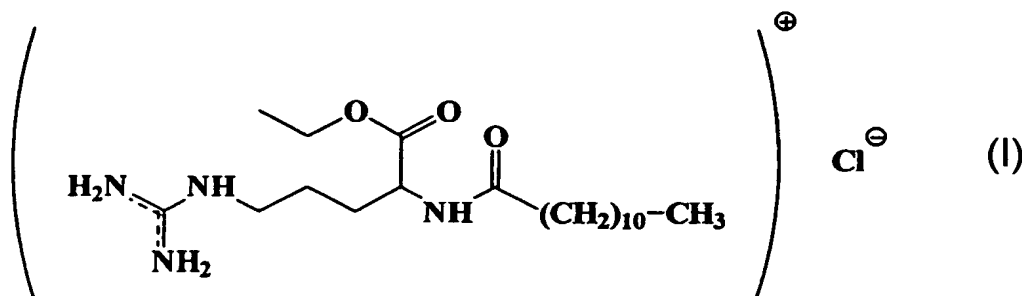
(75) Inventors/Applicants (for US only): **URGELL BEL-**
TRAN, Joan, Baptista [ES/ES]; Calle Cardenal Vives i
Tuto, 37-39 3° 3a, E-08034 Barcelona (ES). **SEGUER**
BONAVENTURA, Joan [ES/ES]; Calle Riera Blanca,
182 5° 2a, E-08902 L'Hospitalet de Llobregat/Barcelona
(ES).(74) Agent: **GILLE HRABAL STRUCK NEIDLEIN PROP**
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(54) Title: USE OF CATIONIC PRESERVATIVE IN FOOD PRODUCTS



(57) **Abstract:** This invention relates to a novel use of cationic preservatives and preparations according to this novel use. A cationic preservative derived from lauric acid and arginine, in particular, the ethyl ester of the lauramide of the arginine monohydrochloride, hereafter named LAE, can be used for the protection against the growth of microorganisms. The chemical structure of LAE is described in formula (I). LAE and related compounds are particularly suitable to be used in the preservation of all perishable food products. It is preferred to dissolve the compound directly before use in one of the following preferred solvents of food grade: water, ethanol, propylene glycol, isopropyl alcohol, other glycols, mixtures of glycols and mixtures of glycols and water. If the treatment shall be performed at a specific pH value the use of a corresponding buffer solution may be recommendable. The composition optionally comprises auxiliary components and excipients. Such auxiliary components and excipients can be thickening agents (e.g. xanthan gum, guar gum, modified starches), anti-foam agents (e.g. dimethylpolysiloxane, silicon dioxide), products to obtain the optimal pH value (e.g. phosphates, tartrates, citrates, lactates), colouring agents (e.g. curcumin, tartrazine, erythrosine), and aroma products. It is preferred, that the preservative composition comprises LAE in an amount of from 0,0001 % to 1 % by weight relative to the whole weight of the preservative composition. It is particularly preferred to use the inventive composition for the preservation of meat products, like for instance meat, poultry products, fish, crustaceans, vegetables, greens, emulsions, sauces, confectionery, bakery, pre-cooked meals, ready-to-serve meals, dairy products, egg-based products, jams, jellies, beverages, juices, wines and beers.

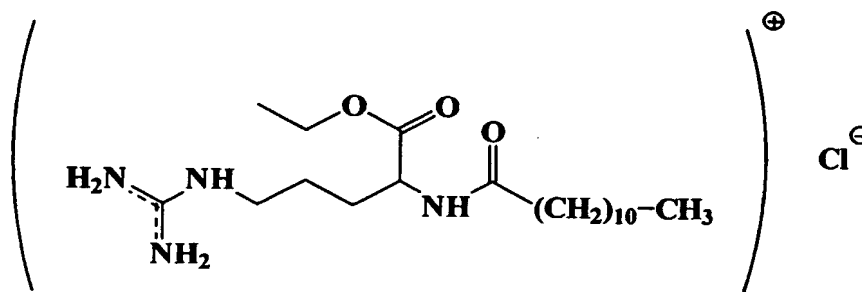
USE OF CATIONIC PRESERVATIVE IN FOOD PRODUCTS

DESCRIPTION

This invention relates to a novel use of cationic preservatives and preparations according to this novel use.

Despite the food industry must avoid the use of preservative products by means of good manufacture practices as it is described in the national and international regulations, it is often necessary to warrant the needed storage capability of the produced food-stuff but never to hide defective effects of a manipulation or manufacture technique.

A cationic preservative derived from lauric acid and arginine, in particular, the ethyl ester of the lauramide of the arginine monohydrochloride, hereafter named LAE, could be used for the protection against the growth of the microorganisms. The chemical structure is described in the following formula:



The preparation of this product has been described in Spanish patent application ES-A-512643.

Biological studies carried out at different research centres under supervision of the present applicant showed LAE to act mainly over the external and cytoplasmatic membrane of the microorganisms and, also, into the cytoplasmatic media, preventing their proliferation. Its action depends on the kind of microorganism and on the exposure time.

Besides, its metabolism in rats has been studied showing a fast absorption and metabolisation into naturally-occurring amino acids and the fatty acid lauric acid, which are eventually excreted as carbon dioxide and urea. Toxicological studies have demonstrated, that LAE is completely harmless to animals and humans.

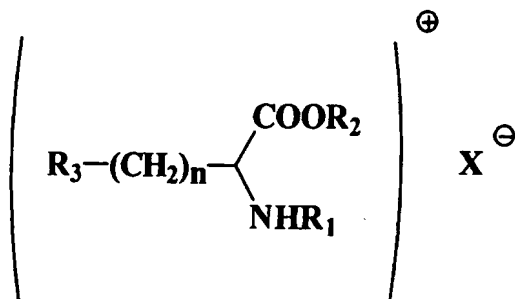
Therefore, LAE and related compounds are particularly suitable to be used in the preservation of all perishable food products.

This compound is remarkable for its inhibitory action over the proliferation of different microorganisms, such as bacteria, fungi and yeasts. The minimum inhibitory concentrations of LAE are shown in the following table 1.

Table 1

Kind	Microorganism	M.I.C. (ppm)
Gram + Bacteria	<i>Arthrobacter oxydans</i> ATCC 8010	64
	<i>Bacillus cereus</i> var <i>mycoide</i> ATCC 11778	32
	<i>Bacillus subtilis</i> ATCC 6633	16
	<i>Clostridium perfringens</i> ATCC 77454	16
	<i>Listeria monocytogenes</i> ATCC 7644	10
	<i>Staphylococcus aureus</i> ATCC 6538	32
	<i>Micrococcus luteus</i> ATCC 9631	128
	<i>Lactobacillus delbrueckii</i> ssp <i>lactis</i> CECT 372	16
	<i>Leuconostoc mesenteroides</i> CECT 912	32
Gram - Bacteria	<i>Alcaligenes faecalis</i> ATCC 8750	64
	<i>Bordetella bronchiseptica</i> ATCC 4617	128
	<i>Citrobacter freundii</i> ATCC 22636	64
	<i>Enterobacter aerogenes</i> CECT 689	32
	<i>Escherichia coli</i> ATCC 8739	32
	<i>Escherichia coli</i> 0157H7	20
	<i>Klebsiella pneumoniae</i> var <i>pneumoniae</i> CECT 178	32
	<i>Proteus mirabilis</i> CECT 170	32
	<i>Pseudomonas aeruginosa</i> ATCC 9027	64
	<i>Salmonella typhimurium</i> ATCC16028	32
	<i>Serratia marcescens</i> CECT 274	32
	<i>Mycobacterium phlei</i> ATCC 41423	2
Fungi	<i>Aspergillus niger</i> ATCC14604	32
	<i>Aureobasidium pullulans</i> ATCC 9348	16
	<i>Gliocadium virens</i> ATCC 4645	32
	<i>Chaetium globosum</i> ATCC 6205	16
	<i>Penicillium chrysogenum</i> CECT 2802	128
	<i>Penicillium funiculosum</i> CECT 2914	16
Yeast	<i>Candida albicans</i> ATCC 10231	16
	<i>Rhodotorula rubra</i> CECT 1158	16
	<i>Saccharomyces cerevisiae</i> ATCC 9763	32

The use of the invention relates to cationic preservatives derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula:



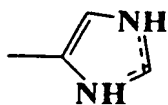
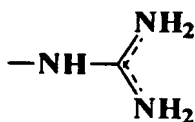
where:

X⁻ is Br⁻, Cl⁻, or HSO₄⁻

R₁: is a linear alkyl chain from a saturated fatty acid or hydroxyacid from 8 to 14 atoms of carbon bonded to the α- amino acid group through an amidic bond.

R₂: is a linear or branched alkyl chain from 1 to 18 carbon atoms or an aromatic group.

R₃ is:



and n can be from 0 to 4.

The most preferred compound of the above class of compounds is LAE.

It is preferred to dissolve the compound directly before use in one of the following preferred solvents of food grade: water, ethanol, propylene glycol, isopropyl alcohol, other glycols, mixtures of glycols and mixtures of glycols and water. If the treatment shall be performed at a specific pH value the use of a corresponding buffer solution may be recommendable.

The composition optionally comprises auxiliary components and excipients. Such auxiliary components and excipients can be thickening agents (e.g. xanthan gum, guar gum, modified starches), anti-foam agents (e.g. dimethylpolysiloxane, silicon dioxide), products to obtain the optimal

pH value (e.g. phosphates, tartrates, citrates, lactates), colouring agents (e.g. curcumin, tartrazine, erythrosine), and aroma products. It is preferred, that the preservative composition comprises LAE in an amount of from 0,0001% to 1% by weight relative to the whole weight of the preservative composition.

It is particularly preferred to use the inventive composition for the preservation of meat products, like for instance meat, poultry products, fish, crustaceans, vegetables, greens, emulsions, sauces, confectionery, bakery, pre-cooked meals, ready-to-serve meals, dairy products, egg-based products, jams, jellies, beverages, juices, wines and beers.

Moreover, the intended use relates to: wine-based flavoured drinks including products; non-alcoholic flavoured drinks; liquid tea concentrates and liquid fruit and herbal infusion concentrates; Barley Water; fruit and citric juices; Capilé Groselha; grape juices, unfermented, for sacramental use; wines, alcohol-free wines, fruit wines (including alcohol-free), alcoholic drinks with fruit; made wines, fruit sparkling wines, ciders, beers and perries (including alcohol-free); fermentation vinager; sod, soft; mead; spirits with less than 15% alcohol by volume; fillings of ravioli and similar products; quince, jams, jellies, marmelades and other fruit based spreads, candied, crystallized and glacé fruit and vegetables; sugar, glucose syrup, molasses and other sugars; transformed and dried fruits and vegetables, Frugtrod and Rote Grütze, fruit and vegetable preparations (including fruit-based sauces); vegetable flesh; shell fruits; mousse, compote; salads, fruits and similar products, canned or bottled; Mostarda Di Fruta; Mascarpone; fruit based cake fillings; fruit gelling extracts and liquid pectine; vegetables and fruits in vinegar, brine or oil; rehydrated dried fruits; dressed dried fruits; sweetcorn canned in vacuum; potato dough and pre-fried, sliced, transformed, frozen, deep-frozen and peeled potatoes; dehydrated potato flakes and granulated (?); gnocchi; polenta; olives and olive-based preparations; jelly coating of meat products (cooked, cured or dried); burger meat; heat-treated meat products, sausages, breakfast sausages, pickled porks, pates, Foie Gras, Foie Gras Entier, Blocs de Foie Gras; Sagu; Mehu and Makeutettu; Ostkaka; Pasha; Semmelknodelteig; Polsebrod and bollery Dansk; canned Flutes; gelatine; collagen based covers with a water activity of more than 0.6; salted meats, cured placenta, dried meat products; semi-preserved fish products including fish roe products, pickling, salted, dried fish, shrimps, cooked, Crangon crangon and Crangon vulgaris cooked; fresh, cooked, frozen and deep-frozen crustacean; cheese, pre-packed, sliced, unripened and cured cheese, processed cheese, layered cheese and cheese with added foodstuffs; superficial treatment of cheese, fruits and vegetables; cheese substitute, meat substitute, fish substitute, crustacean substitute; non-heat-treated dairy-based desserts, curdled milk, semolina and tapioca based desserts; liquid egg (white, yolk or whole egg), dehydrated, concentrated, frozen and deep-frozen egg products; pre-packed and sliced bread and rye-bread; partially baked, pre-packed bakery wares intended for retail sale, fine bakery wares with a water activity of more than 0.65; low-energetic bread; dry-biscuits; cereal- or potato-based snacks and coated nuts; batters, confectionery, glucose syrup based confectionery, flour based confectionery with a water activity of more than 0.65, chewing gum; christmas pudding, nougats and marzipans; clotted cream; toppings (syrups for pancakes, flavoured syrups for milkshakes and ice cream, similar products), fat emulsions, dressing salads, emulsified

sauces, non-emulsified sauces; prepared salads, mustard, seasonings and condiments; liquid soups and broths; aspic, liquid dietary food supplements; pearl barley; dietetic foods intended for special medical purposes and starches; dietetic formulae for weight control intended to replace total daily food intake or an individual meal; and other food products where the use of preservatives became necessary and allowed by law.

The cationic preservative may be added to a final stage of the product to be preserved or it may be added to a initial stage which would have the advantage of treating the food product, whereby it may be added as dry product to the product to be preserved, or in the form of a solution or dispersion.

The food products according to the invention are prepared according to the techniques which are well known to a person skilled in the art.

Procedures to determine the microbiological population and preservative effect

The determination of the microbiological population is carried out according to the ISO standards.

The samples are treated by dilution in buffer peptone with the appropriate neutralising agent of the preservative. The culture media used for counting the microorganisms are: Soya triptone agar (32-35°C, 48 hours) for the determination of mesophilic bacteria; Sabouraud agar with chloramfenicol (25°C, 3-5 days) for fungi and yeast; Violet red bile glucose agar (32-35°C, 24 hours) for enterobacteria; Soya triptone agar (17°C, 5 days) for psychrotrophic bacteria.

EXAMPLES

Different examples of food products and formulations are shown where the product has been assayed. These examples are a part of the preparations and formulations assayed.

- **Example 1:**

This example shows the use of LAE in semi-preserved codfish in oil (table 2). The sample LAE was added to the oil assayed at a concentration of 100 ppm and its microbiological evolution at 4°C was compared against a control.

Table 2

Sample	Time (days)	0		14		43	
	Microorganism	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
	Control (cfu/g)	$3.4 \cdot 10^3$	$4.0 \cdot 10^2$	$3.8 \cdot 10^5$	$2.0 \cdot 10^4$	$2.7 \cdot 10^8$	$1.2 \cdot 10^7$
	With LAE (cfu/g)	$7.6 \cdot 10^3$	$3.0 \cdot 10^2$	$1.0 \cdot 10^4$	$5.4 \cdot 10^3$	$8.5 \cdot 10^4$	$8.2 \cdot 10^3$

▪ **Example 2:**

This example shows the use of LAE in a chicken product (table 3). The sample LAE was added to at a concentration of 150 ppm and the evolution of aerobe and psychrotrophic bacteria at 10°C was compared against a control.

Table 3

Sample	Time (days)	0		14		43	
	Microorganism	Aerobe Bacteria	Psychro. Bacteria	Aerobe Bacteria	Psychro. Bacteria	Aerobe Bacteria	Psychro. Bacteria
	Control (cfu/g)	$3.1 \cdot 10^5$	$2.4 \cdot 10^4$	$9.8 \cdot 10^5$	$6.5 \cdot 10^5$	$7.5 \cdot 10^8$	$4.2 \cdot 10^8$
	With LAE (cfu/g)	$1.2 \cdot 10^5$	$3.0 \cdot 10^4$	$4.2 \cdot 10^5$	$7.1 \cdot 10^4$	$6.1 \cdot 10^5$	$6.8 \cdot 10^4$

▪ **Example 3:**

This example shows the use of LAE in a carbonated orange beverage (table 4). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 17°C was compared against a control.

Table 4

Sample	Time (days)	0		14	
	Microorganism	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
	Control (cfu/g)	$4.0 \cdot 10^2$	<10	$6.5 \cdot 10^4$	$1.7 \cdot 10^3$
	With LAE (cfu/g)	$4.3 \cdot 10^2$	<10	$1.0 \cdot 10^2$	<10

▪ **Example 4:**

This example shows the use of LAE in a blackberry juice (table 5). The sample LAE was added to at a concentration of 60 ppm and its microbiological evolution at 34°C was compared against a control.

Table 5

	Time (days)	0		14	
	Microorganism	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
	Sample				
	Control (cfu/g)	$5.1 \cdot 10^2$	<10	$2.5 \cdot 10^5$	$3.7 \cdot 10^4$
	With LAE (cfu/g)	$4.0 \cdot 10^2$	<10	$2.4 \cdot 10^3$	<10

▪ **Example 5:**

This example shows the use of LAE in custard (table 6). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 25°C was compared against a control.

Table 6

	Time (days)	0		5	
	Microorganism	Aerobe Bacteria	Anaerobe Bacteria	Aerobe Bacteria	Anaerobe Bacteria
	Sample				
	Control (cfu/g)	<10	<10	$9,1 \cdot 10^7$	$3,4 \cdot 10^7$
	With LAE (cfu/g)	<10	<10	$1,1 \cdot 10^3$	$4,1 \cdot 10^2$

▪ **Example 6:**

This example shows the use of LAE in fairy cakes (table 7). The sample LAE was added to at a concentration of 80 ppm and its microbiological evolution at 25°C was compared against a control.

Table 7

	Time (months)	0		3	
	Microorganism	Aerobe Bacteria	Mould & yeast	Aerobe Bacteria	Mould & yeast
	Sample				
	Control (cfu/g)	<10	<10	$9,1 \cdot 10^4$	$3,4 \cdot 10^3$
	With LAE (cfu/g)	<10	<10	$1,1 \cdot 10^2$	<10

▪ **Example 7:**

This example shows the use of LAE in veal stew (table 8). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 10°C was compared against a control.

Table 8

Sample	Time (days)	0			14		
	Microorganism	Aerobe Bacteria	Mould & yeast	Enterobacteria	Aerobe Bacteria	Mould & yeast	Enterobacteria
	Control (cfu/g)	<10	<10	<10	$9,1 \cdot 10^4$	$3,4 \cdot 10^3$	$1,1 \cdot 10^2$
	With LAE (cfu/g)	<10	<10	<10	<10	<10	<10

▪ **Example 8:**

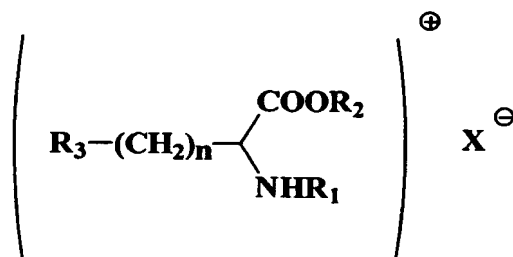
This example shows the use of LAE in ketchup (table 9). The sample LAE was added to at a concentration of 100 ppm and its microbiological evolution at 25°C was compared against a control.

Table 9

Sample	Time (days)	0			14		
	Microorganism	Aerobe Bacteria	Mould & yeast	Enterobacteria	Aerobe Bacteria	Mould & yeast	Enterobacteria
	Control (cfu/g)	<10	<10	<10	$1,2 \cdot 10^6$	$4,3 \cdot 10^2$	$1,4 \cdot 10^3$
	With LAE (cfu/g)	<10	<10	<10	$2,2 \cdot 10^3$	$1,4 \cdot 10^1$	<10

CLAIMS

1. Use of cationic preservatives derived from the condensation of fatty acids and esterified dibasic amino acids, according to the following formula:



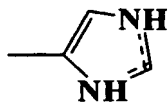
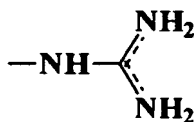
where:

X⁻ is Br⁻, Cl⁻, or HSO₄⁻

R₁: is linear alkyl chain from an saturated fatty acid, or hydroxyacid from 8 to 14 atoms of carbon bonded to the α- amino acid group through amidic bond.

R₂: is a lineal or branched alkyl chain from 1 to 18 carbon atoms or aromatic.

R₃: is:



and n can be from 0 to 4,

as preservatives in food preparations.

2. The use according to Claim 1, wherein the cationic preservative is the ethyl ester of the lauramide of arginine hydrochloride (LAE).
3. Food products comprising the cationic preservative defined in claim 1 or 2 as a preservative.

4. The food products of claim 3, wherein the cationic preservative is LAE at a concentration of from 0,0001% to 1%.
5. Method of preservation of food products wherein LAE is added to such food products at a concentration from 0,0001% to 1%.
6. The method of claim 5 wherein the food products are fish, crustaceans, fish substitutes or crustaceans substitutes.
7. The method of claim 5 wherein the food products are meat, meat substitutes or poultry products.
8. The method of claim 5 wherein the food products are vegetables, greens, sauces and emulsions.
9. The method of claim 5 wherein the food products are beverages, juices, wines or beers.
10. The method of claim 5 wherein the food products are dairy, egg-based, jam, jelly, bakery or confectionery products.
11. The method of claim 5 wherein the food products are pre-cooked meal and ready-to-serve meal products.
12. Method of preservation of food products, wherein LAE is added as a solution, dispersion or solid or is sprayed to a food product before, during and/or after the manufacture.
13. Method of preservation of food products, wherein LAE is applied by surface treatment or mixed in the composition.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/12358

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L3/3526 A23B7/154 A21D15/00 A23L2/44 A23B4/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A23B A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 749 960 A (MIRET LAB) 27 December 1996 (1996-12-27) page 4, line 1 -page 5, line 47; claims 1,2,8,9 ---	1-5,7, 11-13
X	GB 1 352 420 A (AJINOMOTO KK) 8 May 1974 (1974-05-08) page 4, column 1, line 20 -column 2, line 59; claims 1-6,12,14,17,19; examples 6,7 ---	1-13
X	US 3 825 560 A (SAITO T ET AL) 23 July 1974 (1974-07-23) column 7, line 18 - line 34; claims; examples 9-11 --- -/--	1-13

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

18 July 2002

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Baminger, U

INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International Application No

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